Monobind Inc.
Lake Forest, CA 92630, USA

AccuLite
CLIA Microwells

Troponin-I (cTnI) Test System

Product Code: 3875-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Circulating Troponin-I concentrations in Human Serum by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

The cardiac-specific isoforms of Troponin I (cTnI) has been known as a marker of heart damage and myocardial cell death, due to myocardial infarction, for just over 10 years. Troponin-I (cTnI, 24 kDa) is the inhibitory subunit of the Troponin complex of striated muscle. Most of the Troponin (I & T) proteins are located within the contractile apparatus of the striated muscle. The concentration of these subunits is increased in circulation for many days after AMI, because release from the structural elements requires degradation of myofibril itself. This location and release from the specific myocardial tissue and their sustained appearance in circulation make Troponin subunits reliable bio-markers of AMI. Unlike CK-MB and Myoglobin, Troponin does not suffer from non-specificity issues. The immunological determination of cTnI does have some issues surrounding it but those are mainly due to lack of mass standardization, the presence of post-translational modified cTnI in the circulation and variations in the antibody cross-reactivities to the various detectable forms of cTnI. However, cTnI, unlike Myoglobin, has not been found in circulation in marathon runners and other cases of skeletal injury or trauma. Careful selection of antibodies and diligent preparation of stable cTnI for calibration permits cTnI to be an excellent biochemical marker of myocardial damage.

Troponin I (cTnI) is the inhibitory subunit of the Troponin complex, which regulates the calcium modulated interaction of actin and myosin in striated muscle. The complex is a heterodimer consisting of troponins C, I and T, which are tightly bound to the contractile apparatus; hence, circulating concentrations are low. Even though Troponin C and T are both considered to be equally good markers for AMI, the NH_2 terminus of cTnI has 31 additional amino acids not present in the skeletal isoforms and has generated an interest in easily creating cTnI-specific MoAb's, by researchers. Troponin I, however, has its own problems that researchers are dealing with. Troponin I is not very stable. A major portion of it is bound with troponin C (cTnC) and the remaining small part is free in circulation. Again, the post translational modifications, including selective degradation, covalent complex formation and phosphorylation of cTnI in the post-ischemic myocardium complicate the matters further more. Also, the cTnI proteolysis is even more extensive in human myocardium. Some researchers attribute it in part to the heterogeneity of disease states present in a given patient population. The key to overcoming these problems is a very careful selection of antibodies, the matrix for stabilizing the cTnI and the cTnI protein used as the standard itself. Monobind has been able to successfully put together an assay that promises to have overcome those issues.

In this method, Troponin-I calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of Troponin-I are added and the reactants mixed. Reaction between the various Troponin-I antibodies and native Troponin-I forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme- Troponin-I antibody bound conjugate is separated from the unbound enzyme- Troponin-I conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce light. The employment of several serum references of known Troponin-I levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with Troponin-I concentration.

3.0 PRINCIPLE

Sandwich Equilibrium Method (TYPE 2):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of x-cTnI antibody coated on the well.

Upon mixing the enzyme-labeled antibody and a serum containing the native antigen, a reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab + Ag_{cTnI} + Ab_{(well)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{cTnI} - Ab_{(well)}$$

$Ab_{(well)}$ = Antibody coated on well (Excess Quantity)
Ag_{cTnI} = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{cTnI} - Ab_{(well)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

After sufficient time results, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Troponin-I Calibrators – 1.0 ml/vial (Dried) Icons A – F
Six (6) vials of references for Troponin-I antigen manufactured at levels of 0(**A**), 0.4(**B**), 1.25(**C**), 2.5(**D**), 7.5(**E**), and 20(**F**) ng/ml. ***Reconstitute each vial with 1.0ml of distilled or deionized water.***
The reconstituted calibrators are stable for 24 hours at 2-8°C. In order to store for a longer period of time aliquot the reconstituted calibrators in cryo vials and store at -20°C. ***DO NOT FREEZE / THAW MORE THAN ONCE.***
Note: The calibrators, human serum based, were calibrated using NIST standard for cTnI # 2921.

B. cTnI Tracer Reagent – 13 ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled affinity purified antibody and biotin labeled monoclonal mouse anti-troponin IgG in buffer, dye, and preservative. Store at 2-8°C

C. Light Reaction Wells – 96 wells – Icon
One 96-well white microplate coated with x-cTnI antibody and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution (Concentrate) – 20 ml/vial – Icon
One (1) vial containing a surfactant dissolved in buffered saline. A preservative has been added. Store at 2-8°C.

E. Signal Reagent A – 7.0ml/vial – Icon C[A]
One (1) vial containing luminol in buffer. Store at 2-8°C. (See Reagent Preparation section)

F. Signal Reagent B – 7.0ml/vial – Icon C[B]
One (1) vial containing hydrogen peroxide (H_2O_2) dissolved in buffer. Store at 2-8°C. (See Reagent Preparation section)

G. Product Insert.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when sotred at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.025 & 0.100ml (25 & 100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5% (optional).
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Luminometer
5. Container(s) for mixing of reagents (see below).
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Storage container for storage of wash buffer.
11. Distilled or deionized water.
12. Quality Control Materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1&2 and HCV antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. The blood should be collected in a plain redtop venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050 ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated ranges for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied regents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicated unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C.
2. **Working Signal Reagent Solution -** Store at 2 - 8°C.
Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-25°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25µl) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100 ml (100µl) of the Troponin-I Tracer Reagent to each well. **It is very important to dispense all reagents close to the bottom of the microwell.**
Note: *Use a multichannel pipette to quickly dispense Tracer Reagent to avoid drift if the dispensing is to take more than a few minutes.*
4. Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap.
5. Incubate for 15 minutes at room temperature (20-25°C).
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container. Avoiding air bubbles. Decant the wash and repeat four (4) additional times.**
8. Add 0.100 ml (100µl) of working signal reagent to all wells **(see Reagent Preparation Section)**.
DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION
9. Incubate for five (5) minutes at room temperature in the dark.
10. Read the relative light units (RLUs) in each well for 0.2 – 1.0 seconds. **The results should be read within thirty (30) minutes of adding the signal reagent solution.**

NOTE: Always add reagents with multi-channel micropipettes in the same order to minimize reaction time differences between wells.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of troponin in unknown specimens.
1. Record the RLUs obtained from the printout of the microplate luminometer as outlined in Example 1.
2. Plot the light intensity for each duplicate serum reference versus the corresponding troponin concentration in ng/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of troponin for an unknown, locate the average RLUs of the unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLUs

(47800) of control 2 intersects the calibration curve at (10.4ng/ml) troponin concentration (See Figure 1).

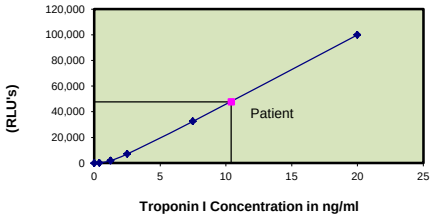
Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	RLUs	Mean RLUs	Value (ng/ml)
Cal A	A1	9	9	0
	B1	8		
Cal B	C1	141	147	0.4
	D1	153		
Cal C	E1	1842	1897	1.25
	F1	1951		
Cal D	G1	7238	7073	2.5
	H1	6908		
Cal E	A2	31431	32517	7.5
	B2	33603		
Cal F	C2	97457	100000	20.0
	D2	102543		
Ctrl 1	E2	78	71	0.32
	F2	64		
Ctrl 2	G2	6015	5970	2.202
	H2	5924		
Pat. 1	A3	48354	47800	10.4
	B3	47245		

* The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLU's for the F calibrator (greatest light output). This conversion eliminates differences cause by efficiency of the various instruments that can be used to measure light output. The conversion varies between instruments and should be established for each instrument before using it as a factor.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available upon request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
9. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
10. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
11. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can occur causing erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays: "Boscato LM, Stuart MC,"Heterophilic antibodies: a problem for all immunoassays", *Clin Chem, 3427-33 (1988).* For diagnostic purposes the results from this assay should be used in combination with clinical examination, patient's history and, all other clinical findings.

13.0 EXPECTED VALUES

Troponin-I values are different in plasma and serum. Plasma sample again may be affected by the additives used. For example heparin does not affect the results but oxalate and EDTA do. A serum sample that has been quickly separated from red cells is preferred.
Based on the clinical data gathered by Monobind in concordance with the published literature the following ranges have been assigned. **These ranges should be used as guidelines only:**

Adult (Normal)	≤ 0.4 ng/ml

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the cTnI AccuLite® CLIA Test System were determined by analyses on three different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

SAMPLE	N	X	σ	CV
Low	20	0.41	0.023	5.7%
Medium	20	3.81	0.090	2.4%
High	20	14.44	0.268	1.9%

TABLE 3
Between Assay Precision (Values in ng/ml)

SAMPLE	N	X	σ	CV
Low	10	0.43	0.032	7.4%
Medium	10	3.94	0.183	4.6%
High	10	13.86	0.662	4.8%

*As measured in ten experiments in duplicate over ten days.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the LoB and LoD. The LoB is 0.0304 ng/ml and the LoD is 0.0853 ng/ml.

14.3 Accuracy

The cTnI AccuLite® CLIA Test System was compared with a reference method. Biological specimens from population were used The values ranged from N/D - 18.1 ng/ml. The total number of such specimens was 74. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This method (x)	2.86	y=0.0553+0.988*(x)	0.989
Reference (y)	2.53		

Only slight amounts of bias between this and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross reactivity of the cTnI AccuLite® CLIA Test System to selected substances was evaluated by adding the interfering substance(s) to a serum matrix at the following concentration(s). The antibody system used did not detect any hemoglobin, CK-MB, TnT or FABP when tested at very high concentrations. The presence of lipemia (25 mg/ml); hemoglobin (4.0 mg/ml) and bilirubin (2.5 mg/ml) did not affect the assay precision.

14.5 Hook Effect

Human serum samples spiked with concentrations up to 10,000 ng/ml of cTnI did not show any hook effect with cTnI AccuLite® CLIA Test System.

15.0 REFERENCES

1. Apple Fred S, Christenson RH, Valdes RJ, Andriak AB, Duh Show-Hong, Feng YJ, Saeed AJ, Johnson Nancy J, Koplen Brenda, Mascotti K, and Wu Alan J, "Simultaneous Rapid Measurement of Whole Blood Myoglobin, Creatine Kinase MB, and Cardiac Troponin I by the Triage Cardiac Panel for Detection of Myocardial Infarction", *Clin Chem*, 45, 199-205 (1999).
2. Adams JE, Schechtman KB, Landt Y, et al, "Comparable detection of acute Myocardial infarction by creatine kinase MB isoenzymes and cardiac troponin I", *Clin Chem,* 40,1291-5 (1994).
3. Apple FS, Preese LM, "Creatine kinase-MB: detection of Myocardial infarction and monitoring reperfusion", *J Clin Immunoassay*,17, 24-9 (1994).
4. Bhayana V, Cohoe S, Leung FY, et al, "Diagnostic evaluation of creatine kinase-2 mass and creatine kinase-3 and -2 isoform ratios in early diagnosis of acute Myocardial infarction", *Clin Chem,* 39, 488-95 (1993).
5. Bruns DE, "Diagnosis of acute Myocardial infarction when skeletal muscle damage is present: a caveat regarding use of creatine kinase isoenzymes", *Clin Chem*, 35, 705 (1989).
6. Gibler WB, Lewis LM, Erb RE, et al, "Early detection of acute Myocardial infarction in patients presenting with chest pain and nondiagnostic ECGs; serial Troponin-I sampling in the emergency department", *Ann Emerg Med*, 19, 1359-66 (1990).
7. Henderson AR, Stark JA, McQueen MJ, et al, "Is determination of creatine kinase-2 after electrophoretic separation accurate?", *Clin Chem,* 40, 177-83 (1994).
8. Kallner A, Sylven C, Brodin U, et al, "Early diagnosis of acute Myocardial infarction; a comparison between chemical predictors", *Scand J Clin Lab Invest*, 49, 633-9 (1989).
9. Kiyasu Y, "Current status of detecting Troponin-I for patient management", *Am Clin Prod Rev*, 4, 29-31(1985).
10. Lang H, Wuerzburg U, "Creatine kinase, an enzyme of many forms", *Clin Chem*, 28, 1439-47 (1982).
11. Lee KN, Csako G, Bernhardt P, Elin RJ, "Relevance of macro creatine kinase type 1 and type 2 isoenzymes to laboratory and clinical data", *Clin Chem*, 40, 1278-83 (1994).
12. Lee TH, Rouan GW, Weisberg MC, et al, "Sensitivity of routine clinical criteria for diagnosing Myocardial infarction within 24 hours of hospitalization", *Ann Intern Med*, 106, 181-6 (1987).
13. Panteghini M, "Creatine kinase MB isoforms", *J Clin Immunoassay*, 17, 30-4 (1994).
14. Gibbler WB, Runyon JP, Levy RC, Sayre MR, Kacich R, Hattemar CR, et al, "A rapid diagnostic and treatment center for patients with chest pain in the emergency department", *Ann Emerg Med,* 25, 1-8 (1995).

Effective Date: 2019-Jan-01 Rev. 4 **DCO: 1320**
MP3875 **Product Code: 3875-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)

For Orders and Inquires, please contact
Monobind Inc.
100 North Pointe Drive
Lake Forest, CA 92630 USA
Tel: +1 949.951.2665 Mail: info@monobind.com
Fax: +1 949.951.3539 Fax: www.monobind.com

IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device	2°-8° Temperature Limitation Storage Condition (2-8°C)	Consult Instructions for Use
REF Catalogue Number	Σ Contains Sufficient Test for Σ	LOT Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP Authorized Rep in European Country	CE European Conformity	